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CLASSICAL PAH FORM A MINOR PORTION OF MUTAGENS DETECTED IN HAMILTON AIR PARTICULATES.

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INTRODUCTION

The objective of this research programme is to examine the potential health hazard presented by PAH and PAH derivatives adsorbed to respirable urban particulate. To achieve this goal, we are using a calss sensitive chemical fractionation procedure and an analysis of the fractions by a combination of chromatographic methods and mutagenicity assays. This approach focuses attention on the genotoxic activity of each fraction then on the compounds within that fraction that are responsible for that genotoxic activity.

Chemical analyses of organic material associated with air particulates have produced a bewilderingly complex picture of the composition of urban air. The chemical analysis of organic extracts of total suspended respirable particulate has been extensively reported (Butler et al., 1987; Hoff and Chan, 1987; Yamauchi and Handa, 1987; Daisey et al., 1986; Greenberg et al., 1985). By comparison, suprisingly few of the chemicals identified have been examined using short term tests (STTs). It must be pointed out that not all chemicals which are known to be carcinogenic are detected by all or indeed any of the STTs. Indeed, the analysis of organic extracts of urban air particulate employing STTs have met with limited success (Butler et al., 1987; Pyysalo et al., 1987; Wolff et al., 1986). Application of biological assays for genotoxic activity to total extractable material is generally crude and uninformative. While these biological techniques can be extremely sensitive with individual compounds, their behavior with complex mixtures does not match analytical chemical methodologies.

MATERIALS AND METHODS

Air particulates were collected on 8 x 10 inch galss fiber filters using a hi-vol air sampler (General Metal Works L-2000 H) with flow controller set at 40 ft^3/min (1630 m^3/24h) Samplers were located on the roof of an OME station located near the centre of downtown Hamilton. Particulate samples were acquired over a period between April 4, and May 31, 1988. This period was characterized by frequent atmospheric inversions. Filters were weighed and stored at -80°C until extraction. Filters (22) from eleven days with loadings ranging from 200 to 360 milligrams per 24h (total: 5.528 g) were pooled and extracted with dichloromethane (DCM) and methanol (MEOH) in a soxhlet apparatus for 16h. The organic extract was fractionated according to the scheme presented in Figure 1. Pooled DCM and MeOH extract was applied to a neutral alumina column. Sequential elution with solvents of increasing strength yielded the following fractions: 60 mL hexane [A1], 50 mL benzene [A2], 70 mL cloroform with 1% methanol [A3], 50 mL methanol [A4], 50 mL 4:1 methanol/water [A5], and 50 mL water [A6]. Fractions [A2] and [A3] as well as [A4] and [A5] were pooled to yield [A23] and [A45]. Aliphatics

which were a major contaminant of [A23] were removed by a chromatographic step elution from Sephadex LH20. Additional details of chromatography are given in the figure legends.

The Salmonella typhimurium/microsome assays for mutagenic activity with or without Araclor 1254 induced rat liver S9 (4%) were performed as described by Maron and Ames (1983).

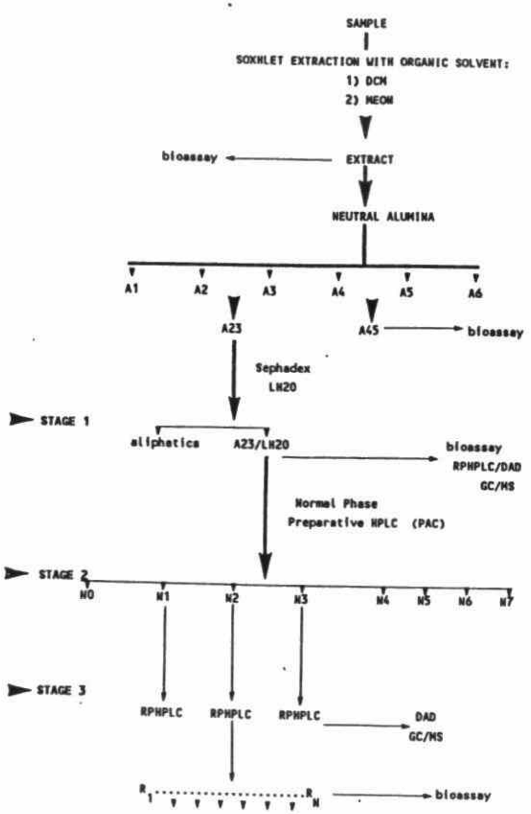

FIGURE 1 The scheme used for fractionation of organic extracts of urban air particulates.

RESULTS

Table 1 shows the mutagenic activity found in the crude alumina fractions from the pooled May filters. Activity, expressed as revertants per m^3 air, was determined from dose response curves. Detectable mutagens were divided between the two pooled eluant fractions A23 and A45. No mutagenic activity was lost after the passage of A23 through Sephadex LH20 (a step used to remove non-mutagenic aliphatics). Direct acting mutagens (-S9) were about equally divided between these two fractions (A23LH20: 51.1%; A45: 46.3%), while activity requiring the addition of rat liver microsomal activation (+S9) was largely found in fractions eluted with less polar solvent (A23LH20: 78.6%; A45: 21%). These results clearly indicate that while much of the mutagenic activity is associated with classical PAH (A23LH20), we have found a significant quantity of material which may be characterized as polar PAH derivatives.

TABLE 1 Mutagenic Activity of Neutral Alumina Fractions: Slope of Linear Regression of Dose Response Curves

FRACTION	Revertants per m^3 Air			
	TA98-S9	TA98+S9	TA100-S9	TA100+S9
A1	0.15	0.13	ND[a]	ND
A23LH20	5.31	26.00	2.2	27.66
A45	4.81	6.95	1.65	5.11
A6	0.12	ND	ND	ND

[a] ND: Activity not detected (background)

The A23LH20 pooled material was further divided into eight fractions (N0..N7) by normal phase chromatography as shown in Figure 2. The selection of cut points for N fractions was based both on the distribution of mutagenic activity and on chemical class. N1 = PAH, polyaromatic furans and polycyclic sulphur compounds; N2 = nitro-PAH and oxy-PAH; N3 = dinitro-PAH and polyaromatic quinones; N4 to N6 = polyaromatic ketones and polyaromatic quinones; N7 = polyaromatic nitrogen compounds (McCalla et al. 1988). Figure 2C shows the distribution of mutagens in these fractions. Column eluant was pooled for each fraction and assayed for mutagens. N1, N2 and N7 all showed a greater mutagenic response with microsomal activation while the remainder were about equally divided between direct and indirect activity. Comparing the proportion of the total mutagenic activity found across the chromatogram, N3 with 54% of the direct acting (-S9) and 41% of the indirect acting (+S9) mutagens had the majority of detected activity. This was followed by N2 (11% [-] & 13% [+]), N6 (12% & 9%), N4 (14% & 6%), N7 (3% & 10%), N5 (5% & 3%) and N1 (0% & 13%). No significant mutagens were found in N0.

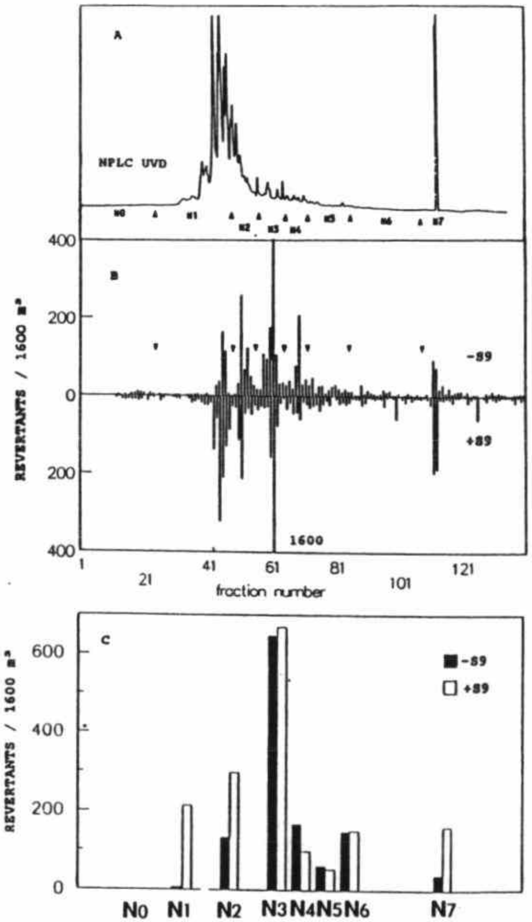

FIGURE 2 Normal phase HPLC analysis of A23LH20 fraction on a Whatman M9-PAC column. Gradient: hexane to 100% CH_2Cl_2 over 30 min; hold 100% CH_2Cl_2 to 50 min, then 0-100% ETOH to 55 min. Panel A: UV detection (250nm-400nm). Panel B: The eluant from NPLC analysis was collected in 0.5 min fractions. Solvent was evaporated and the residue taken up in DMSO for mutagenic assay in S.typhimurium TA98. Each fraction was assayed with (+S9) and without (-S9) microsomal activation. Panel C: Eluant from NPLC analysis was collected in 8 fractions numbered N0..N7 as indicated by the arrows in A. Solvents were evaporated and a portion of each residue analyzed for mutagens in TA98 as indicated on the figure.

Reverse phase chromatography of the N2 fraction of A23LH20 is shown in Figure 3. Here, the mutagens were divided between those with both direct and indirect activity and the more polar mutagens which only expressed genotoxicity with microsomal activation. It is also noteworthy that the less polar mutagens would not be detected if uv absorbance (250-400nm) were the criterion for selection. This also implies that while some of the compounds of the N2 class were not abundant, they possessed significant genotoxic potential.

Additional data including mutachromatograms of fraction N3 of A23LH20, and A45 from May '88 will be presented at the conference. We will also present preliminary data on particulates collected during the summer.

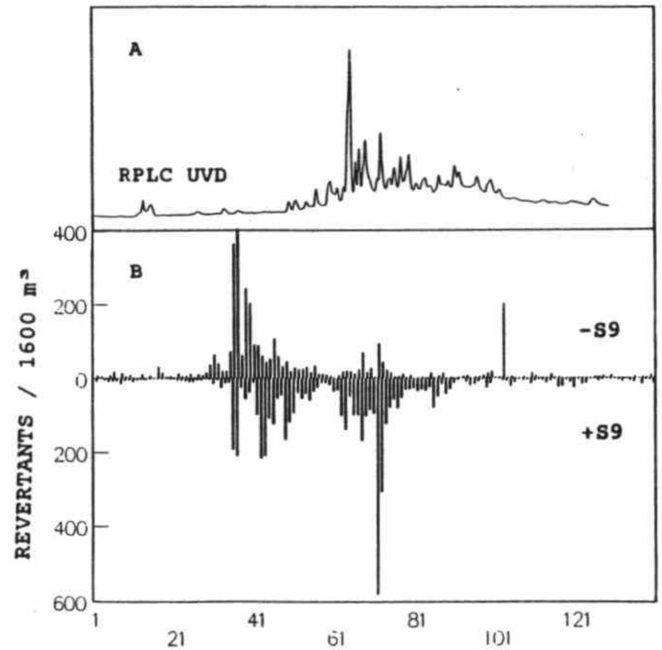

FIGURE 3 Reverse phace HPLC analysis of Fraction N2 on two 25 cm x 0.46 cm Vydac 201TP columns linked in tandem. Gradient: 60% acetonitrile (ACN)/water to 100% ACN over 30 min and hold at 100% ACN. Panel A: UV detection. Panel B: The eluant from RPHPLC analysis was collected in 0.5 min fractions and analyzed for mutagenicity as in Figure 2B.

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